ORIGINAL ARTICLE

Effects of a novel selective peroxisome proliferator-activated receptor-α modulator, pemafibrate, on hepatic and peripheral glucose uptake in patients with hypertriglyceridemia and insulin resistance

Ikuro Matsuba[1]* [iD], Ren Matsuba[2], Shun Ishibashi[3], Shizuya Yamashita[4,5], Hidenori Arai[6], Koutaro Yokote[7], Hideki Suganami[8], Eiichi Araki[9]

[1]Matsuba Clinic, [2]Department of Internal Medicine, Division of Metabolism and Endocrinology, St. Marianna University School of Medicine, Kanagawa, [3]Division of Endocrinology and Metabolism, Department of Medicine, Jichi Medical University, Tochigi, [4]Department of Community Medicine and Department of Cardiovascular Medicine, Osaka University Graduate School of Medicine, [5]Rinku General Medical Center, Osaka, [6]National Center for Geriatrics and Gerontology, Aichi, [7]Department of Clinical Cell Biology and Medicine, Chiba University Graduate School of Medicine, Chiba, [8]Clinical Data Science Department, Kowa Company, Ltd., Tokyo, and [9]Department of Metabolic Medicine, Faculty of Life Sciences, Kumamoto University, Kumamoto, Japan

ABSTRACT

Aims/Introduction: Pemafibrate is a novel selective peroxisome proliferator-activated receptor-α modulator with potent triglyceride-lowering and high-density lipoprotein cholesterol-raising effects. We showed that pemafibrate decreased the homeostatic model assessment for insulin resistance in patients with dyslipidemia. To investigate how pemafibrate improves insulin sensitivity, we used a hyperinsulinemic-euglycemic clamp technique to determine the splanchnic and peripheral glucose uptake in patients with hypertriglyceridemia and insulin resistance.

Materials and Methods: A total of 27 patients with hypertriglyceridemia and insulin resistance were randomly assigned to receive pemafibrate (0.4 mg/day, b.i.d.) or placebo treatment for 12 weeks. The hyperinsulinemic-euglycemic clamp test combined with oral glucose loading was carried out at weeks 0 and 12 to evaluate the splanchnic and peripheral glucose uptake.

Results: Pemafibrate, but not the placebo, significantly increased the splanchnic glucose uptake rate from baseline (19.6 ± 5.9% with $P = 0.005$ and 2.1 ± 7.4% with $P = 0.78$, respectively), although no significant difference between the groups was observed ($P = 0.084$). Conversely, peripheral glucose uptake rate was not significantly altered. Pemafibrate, compared with the placebo, significantly decreased plasma triglycerides (−61.4 ± 16.4% vs −2.5 ± 41.4%, $P = 0.001$), free fatty acids (−24.8 ± 23.2% vs 2.0 ± 26.8%, $P = 0.016$) and gamma-glutamyl transpeptidase (−30 ± 46 vs 10 ± 19 U/L, $P = 0.009$) levels, and significantly increased fibroblast growth factor 21 (457.7 ± 402.1 vs −41.7 ± 37.4 pg/mL, $P = 0.007$) levels.

Conclusions: Pemafibrate increased splanchnic glucose uptake from baseline in patients with hypertriglyceridemia.

INTRODUCTION

Insulin resistance is an important risk factor for the development of type 2 diabetes mellitus and cardiovascular diseases[1,2]. The presence of insulin resistance is indicated by an elevated

homeostatic model assessment for insulin resistance (HOMA-IR) score, and is associated with high triglyceride (TG) and low high-density lipoprotein cholesterol (HDL-C) levels[2,3].

Peroxisome proliferator-activated receptor (PPAR) agonists are used for the treatment of dyslipidemia or type 2 diabetes. PPARs are ligand-activated transcription factors of the nuclear hormone receptor superfamily, which is composed of three subtypes, including PPARα, PPARδ and PPARγ[4]. PPARγ is highly expressed in adipose tissues, and PPARγ agonists sensitize insulin action in both the liver and skeletal muscles, and lower plasma glucose in type 2 diabetes[5–9]. PPARδ is ubiquitously expressed in multiple tissues, including the liver and skeletal muscles[4], and plays an essential role in mitochondrial function, fatty acid (FA) oxidation and insulin sensitivity[10–12]. PPARα is highly expressed in multiple tissues with high FA oxidation capacity, including the liver, kidneys, heart, skeletal muscles and brown adipose tissues[4]. Additionally, PPARα agonists, also known as fibrates, stimulate FA oxidation, suppress FA and TG synthesis, and reduce plasma TG or TG-rich lipoprotein levels[13]. However, previous studies showed inconsistent results regarding the effects of PPARα agonists on glucose metabolism[5,10,14–16].

Pemafibrate (K-877) has a more potent and selective stimulatory effect on PPARα than fenofibrate[17,18]. We have recently shown that pemafibrate treatment resulted in a significant plasma TG decrease and HDL-C increase in patients with dyslipidemia with less adverse effects, such as increased serum creatinine and liver enzyme[19], which are commonly observed and associated with fenofibrate treatment[20]. Therefore, we designated pemafibrate as a selective PPARα modulator (SPPARMα) with higher efficacy and safety than other agonists[17,18]. Interestingly, pemafibrate decreased fasting insulin levels and HOMA-IR in patients with hypertriglyceridemia[19,21]. It was unclear if their glucose tolerance was normal or not in the pemafibrate study; however, HOMA-IR was dependent on hepatic rather than peripheral insulin sensitivity in individuals with impaired fasting glucose/impaired glucose tolerance in another study[22]. Furthermore, liver enzyme levels significantly decreased with pemafibrate treatment[19], suggesting its beneficial effects on the liver, which plays a key role in the homeostasis of blood glucose. Therefore, we carried out the hyperinsulinemic-euglycemic clamp study combined with oral glucose loading (OGL) to investigate how pemafibrate improves insulin sensitivity in patients with hypertriglyceridemia and insulin resistance. Plasma free fatty acid (FFA), adiponectin, and fibroblast growth factor 21 (FGF21) levels were examined to investigate whether they are correlated with the effects of pemafibrate on insulin sensitivity[23–25].

METHODS

Study design and participants

The present study was an exploratory pharmacology clinical trial carried out in a randomized, parallel-group, placebo-controlled, double-blind design at Matsuba Clinic, Kanagawa, Japan, between December 2013 and March 2015, in accordance with the principles of the Declaration of Helsinki and in compliance with the Good Clinical Practice Guidelines issued by Japan's Ministry of Health, Labor and Welfare (clinical trial registration ID: JapicCTI-142410, registered on 10 January 2014). The study protocol was approved by the institutional review board. All patients were fully informed of the nature of the study and provided written consent before their participation. Men aged ≥20 years and postmenopausal women with fasting TG levels between ≥200 and ≤500 mg/dL (≥2.26 and ≤5.65 mmol/L) and a HOMA-IR score between >1.6 and ≤4.0 were eligible. The following exclusion criteria applied: type 1 diabetes, poorly controlled type 2 diabetes (glycated hemoglobin ≥8.0%), poorly controlled hypertension (≥160 mmHg systolic blood pressure or ≥100 mmHg diastolic blood pressure), poorly controlled thyroid disorder, current or past history of hepatic impairment and aspartate aminotransferase or alanine aminotransferase (ALT) levels over threefold the upper limit of the normal range. The HOMA-IR upper limit for the eligibility was initially set at ≤3.0, because we were concerned about the possibility of underestimating splanchnic glucose uptake (SGU), calculated using glucose infusion rates (GIR) described below, in patients with severe insulin resistance, as it was assumed difficult to maintain GIR >0 after OGL because of the potential impairment of SGU. However, we did not encounter such problems in the evaluation of patients with HOMA-IR of approximately 3.0. Hence, the upper limit was raised to 4.0 during the study.

The study comprised a washout period, a screening period of up to 8 weeks and a treatment period of 12 weeks. During the study, the use of therapeutic agents for dyslipidemia and those with possible effects on insulin sensitivity, such as antidiabetic agents, angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, diuretics and β-blockers, were prohibited. Subsequently, eligible patients were randomly assigned to receive either pemafibrate (0.4 mg/day, b.i.d.) or placebo in a 2:1 ratio, to increase the probability of detecting the change in SGU rate from baseline, using a permuted block method with a block size of 6 after the screening period. An independent third party generated the random allocation sequence, confirmed that the study drugs were indistinguishable, and carried out the numbering and concealment of the drugs. All patients, investigators and the study sponsor were blinded to the treatment assignment during the study period. The study drugs were administered orally every morning and evening after meals for 12 weeks. The hyperinsulinemic-euglycemic clamp test was carried out at weeks 0 and 12, and laboratory examinations were carried out at weeks 0, 4, 8 and 12. Adverse events were recorded throughout the study period.

Clinical laboratory analysis

Fasting blood and urine samples were collected from patients at each attendance for clinical laboratory testing. The samples at weeks 0 and 12 were collected before the clamp test and the

administration of pemafibrate or placebo. Blood was also drawn before the OGL and after the clamp test. The lipoprotein cholesterol and apolipoprotein (Apo) levels were measured using direct enzymatic and immunoassay methods, respectively. Other common laboratory parameters were analyzed through standardized laboratory methods, except for FGF21, which was examined using an enzyme-linked immunosorbent assay with human FGF21 enzyme-linked immunosorbent assay (BioVendor R&D, Brno, Czech Republic). All measurements were carried out by the LSI Medience Corporation (Tokyo, Japan).

Hyperinsulinemic-euglycemic clamp test combined with OGL

The SGU and peripheral glucose uptake (PGU) rates were assessed through the hyperinsulinemic-euglycemic clamp technique combined with OGL. The hyperinsulinemic-euglycemic clamp technique, which was developed by DeFronzo et al.[26,27], has been used as the gold standard method in the evaluation of insulin sensitivity. With this method, endogenous glucose production or hepatic glucose production (HGP) can be measured using 3-[³H]glucose, and hepatic glucose uptake can be measured in combination with OGL using a hepatic venous catheter. Kawamori et al.[6,28–31] developed a modified clamp method combined with OGL to non-invasively evaluate SGU, in addition to PGU, without the use of a tracer and hepatic venous catheterization on the basis that the liver plays a key role in the homeostasis of blood glucose in the post-absorptive state through insulin-mediated hepatic glucose uptake, as well as endogenous glucose production[32]. The modified clamp test was used in the present study and carried out at weeks 0 and 12, after confirmation that the participants had been fasting for >10 h since their last meal. The clamp test at week 12 was commenced after the administration of the study drug in the morning. The insulin infusion rates and GIR were controlled using the artificial pancreas STG-55 (Nikkiso Co., Ltd., Shizuoka, Japan), an upgraded model of STG-22 that has been widely used in Japan since 1987. The plasma glucose levels during the clamp test were determined every minute through the continuous glucose monitoring system in the STG-55 at a sampling rate of 2 mL/h.

PGU Evaluation

A hyperinsulinemic state was achieved through primed-constant insulin infusion at a rate of 17.9 pmol/kg/min, which corresponds to approximately 695 pmol/m²/min, given the average Japanese physique. This was considered to be the rate to achieve a steady-state plasma insulin (SSPI) level of 1,390 pmol/L, which could almost completely suppress HGP, even in patients with insulin resistance[31,33]. To attain the goal of maintaining a steady-state plasma glucose level of approximately 95 mg/dL (5.3 mmol/L), GIR was automatically controlled using STG-55, depending on the plasma glucose levels determined using its continuous glucose monitoring system.

After the achievement of a steady-state GIR, PGU was determined based on the mean GIR for 30 min, which was obtained approximately 90–120 min after the initiation of the insulin infusion. The mean GIR was divided by the SSPI and multiplied by 100 for convenience, and used as another PGU index to adjust for the variation in the individual SSPI level.

SGU Evaluation

After the measurement of the mean GIR, OGL was carried out to evaluate the SGU through oral administration of basically 0.3 g/kg glucose at weeks 0 and 12. After OGL, GIR was accordingly decreased to maintain the plasma glucose levels within a euglycemic range. The clamp test was continued until the GIR returned to its steady-state level before OGL, thus maintaining a steady-state plasma glucose level of approximately 5.3 mmol/L. The integrated GIR reduction, which reflects the glucose amount that was absorbed but not taken up by the liver, was subtracted from the amount of ingested glucose (OGL). The resulting values were divided by OGL and used as the SGU rate, that is: $(OGL - \sum \Delta GIR) / OGL \times 100$ (%).

Statistical analysis

The primary and secondary efficacy end-points were the changes from baseline in the SGU and PGU rates, respectively, measured at week 12. The primary safety end-points were the incidence rates of adverse events and adverse drug reactions. We analyzed the primary and secondary efficacy end-points using an analysis of covariance (ANCOVA) model with the baseline value as a covariate. Other laboratory test parameters were also analyzed using ANCOVA as post-hoc analysis. The continuous and categorical data of the baseline characteristics were analyzed using two-sample t-test and Fisher's exact test, respectively. The reported P-values were two sided, and a value of 0.05 was considered to be significant. All randomized participants who received at least one dose of the study drug were included in the safety-analysis set, which was for primary safety analyses. Among them, those who had valid baseline and post-baseline measurements for the primary and secondary efficacy end-points without protocol deviations that can affect the efficacy evaluation were included in the per-protocol set, which was for primary efficacy analyses. The statistical power calculation was based on the results of a study that evaluated the effect of pioglitazone on insulin resistance in patients with non-insulin-dependent diabetes mellitus[6]. In the pioglitazone study, the change from baseline in SGU rate was 30.8 ± 34.4% and −0.9 ± 21.0% in the pioglitazone and placebo group, respectively. Therefore, between-group difference in this parameter was assumed to be 30 ± 30%, or potentially 30 ± 20%, in the present study. Approximately 30 patients were considered as a feasible sample size for the present study. The estimated power was 60% with the difference in SGU rate of 30 ± 30%, but 91.1% with that of 30 ± 20%, in the setting with 24 patients composed of 16 in the pemafibrate and eight in the placebo group. Because the present study was an exploratory clinical pharmacology study, we set the target number of participants

as 24 (16 in pemafibrate, 8 in placebo). All analyses were carried out using SAS version 9.3 software (SAS Institute Inc., Cary, NC, USA). The primary and secondary efficacy and primary safety end-points were analyzed in accordance with a pre-specified statistical analysis plan.

RESULTS

Recruitment and treatment of participants

Among 44 patients who provided informed consent, 27 patients were eligible and randomly assigned to either the placebo ($n = 8$) or pemafibrate ($n = 19$) group. All of the 27 patients were included in the safety-analysis set, and 18 patients (7 and 11 in the placebo and pemafibrate groups, respectively) were included in the per-protocol set because of protocol deviations in nine patients, such as consent withdrawal ($n = 1$), discontinuation due to the occurrence of adverse events ($n = 2$), extremely low TG levels at week 0 ($n = 1$) or lack of paired measurements to calculate the change in glucose uptake rates due to failure of adhering to the hyperinsulinemic-euglycemic clamp test protocol ($n = 5$).

Baseline characteristics

Tables 1 and 2 show the baseline characteristics and clinical laboratory test results, respectively. Although men, post-menopausal women and patients with type 2 diabetes were eligible for the study, all the patients were men without type 2 diabetes. The mean values for the baseline parameters were as follows: age 50 years; body mass index 25 kg/m²; fasting TG 3.30 mmol/L; fasting plasma glucose 5.66 mmol/L; and fasting insulin 83 pmol/L. All characteristics were comparable between the groups.

Glucose clamp test

The SSPI levels before OGL during the clamp test in the placebo and pemafibrate groups were $1,714 \pm 438$ and $1,741 \pm 240$ pmol/L, respectively, at week 0, and $1,928 \pm 427$ and $1,757 \pm 397$ pmol/L, respectively, at week 12. These were >1,390 pmol/L, the level at which the HGP can be almost completely suppressed, even in patients with insulin resistance. The SGU rates significantly increased from baseline in the pemafibrate group (19.6%, $P = 0.005$), but not in the placebo group (2.1%, $P = 0.78$; Figure 1a). No significant difference between

groups was observed ($P = 0.084$). Furthermore, the changes in the mean GIR and GIR/SSPI from baseline and the differences between the groups were not significant (Figure 1b,c).

Major laboratory data

Table 2 and Figure 2 present the major laboratory test results. The TG, ApoCIII and FFA levels significantly decreased, whereas HDL-C, ApoAI and ApoAII levels significantly increased in the pemafibrate group compared with the placebo group. The glycemic parameters were not altered at week 12; however, the glycoalbumin level significantly decreased at weeks 4 and 8 in the pemafibrate group compared with the placebo group (Figure 2c). A similar trend was observed in the fasting plasma glucose and fasting insulin levels over the study period (Figure 2a,b). Further parameters showed significant changes at week 12: the FGF21 level increased (Figure 2e) and gamma-glutamyl transpeptidase level decreased throughout the study period (Figure 2f). The ALT level also decreased, although this change was not statistically significant. Several correlations between the SGU or PGU rate and laboratory parameters were found to be significant, but were not clinically meaningful (data not shown).

Adverse events

No serious adverse event was observed during the study period. The incidence rates of adverse events were 25.0% (2/8) and 10.5% (2/19) in the placebo and pemafibrate groups, respectively. Six adverse events were observed in four patients, which included arthropod sting and urticaria ($n = 1$), increased blood creatine phosphokinase level, and abnormal liver function test ($n = 1$) in the pemafibrate group, and hypoglycemia ($n = 1$) and upper respiratory tract inflammation ($n = 1$) in the placebo group. A causal relationship between pemafibrate treatment and increased creatine phosphokinase level and abnormal liver function test could not be excluded. Hypoglycemia observed in the placebo group was attributed to the hyperinsulinemic-euglycemic clamp test process. Participation in the study was discontinued for two patients in the pemafibrate group due to the occurrence of adverse events: increased creatine phosphokinase level and abnormal liver function tests ($n = 1$), and urticaria ($n = 1$). All patients who experienced adverse events recovered within 14 days.

Table 1 | Baseline characteristics of the participants

	Placebo ($n = 7$)	Pemafibrate ($n = 11$)	*P*-value (vs placebo)
Age (years)	46.4 ± 7.7	51.6 ± 10.4	0.27
Body mass index (kg/m²)	25.8 ± 2.9	25.2 ± 2.3	0.60
Waist circumference (cm)	90.8 ± 6.5	89.0 ± 6.8	0.58
Male	7 (100)	11 (100)	–
Current smoker	3 (42.9)	3 (27.3)	0.23
Hypertension	0	2 (18.2)	0.50

Data are presented as mean ± standard deviation and *n* (%) for continuous and categorical values, respectively.

Table 2 | Laboratory test results

	Placebo (n = 7)	Pemafibrate (n = 11)	P-value (vs placebo)
TG (mmol/L)			
Baseline	3.07 ± 0.60	3.45 ± 1.10	
Week 12	2.99 ± 1.34	1.24 ± 0.38	
% Change	−2.5 ± 41.4	−61.4 ± 16.4	0.001
HDL-C (mmol/L)			
Baseline	1.08 ± 0.12	1.08 ± 0.22	
Week 12	1.07 ± 0.16	1.34 ± 0.31	
% Change	−0.4 ± 12.5	24.2 ± 15.6	0.004
Non-HDL-C (mmol/L)			
Baseline	4.86 ± 0.65	4.43 ± 1.08	
Week 12	5.08 ± 0.89	3.92 ± 0.91	
% Change	4.2 ± 7.3	−9.6 ± 20.0	0.060
ApoAI (mg/dL)			
Baseline	123 ± 12	122 ± 18	
Week 12	127 ± 15	143 ± 21	
% Change	3.3 ± 9.7	17.9 ± 12.1	0.018
ApoAII (mg/dL)			
Baseline	30.5 ± 2.5	28.6 ± 4.5	
Week 12	29.9 ± 4.2	40.5 ± 5.5	
% Change	−2.1 ± 10.8	42.7 ± 13.6	<0.001
ApoB (mg/dL)			
Baseline	108 ± 16	95 ± 22	
Week 12	110 ± 18	91 ± 19	
% Change	1.6 ± 7.9	−3.1 ± 16.8	0.238
ApoCIII (mg/dL)			
Baseline	15.8 ± 3.6	16.1 ± 5.8	
Week 12	14.9 ± 4.4	8.8 ± 2.9	
% Change	−7.1 ± 15.6	−42.3 ± 19.1	0.001
FFA (mEq/L)			
Baseline	0.85 ± 0.30	0.69 ± 0.19	
Week 12	0.84 ± 0.24	0.51 ± 0.15	
% Change	2.0 ± 26.8	−24.8 ± 23.2	0.016
HbA$_{1c}$ (%)			
Baseline	5.6 ± 0.2	5.6 ± 0.2	
Week 12	5.7 ± 0.3	5.8 ± 0.3	
Change	0.1 ± 0.1	0.2 ± 0.2	0.270
Glycoalbumin (%)			
Baseline	12.4 ± 1.3	12.8 ± 1.1	
Week 12	12.4 ± 1.2	12.5 ± 1.3	
Change	0.0 ± 0.5	−0.3 ± 0.5	0.233
FPG (mmol/L)			
Baseline	5.58 ± 0.12	5.68 ± 0.49	
Week 12	5.61 ± 0.24	5.65 ± 0.95	
Change	0.03 ± 0.18	−0.03 ± 0.66	0.691
Fasting insulin (pmol/L)			
Baseline	85.0 ± 28.4	79.3 ± 38.5	
Week 12	112.9 ± 76.4	105.7 ± 147.1	
Change	27.9 ± 55.6	26.4 ± 113.8	0.730
HOMA-IR			
Baseline	3.1 ± 1.1	3.0 ± 1.8	
Week 12	4.1 ± 2.8	4.6 ± 8.0	
Change	1.0 ± 2.0	1.6 ± 6.3	0.611

Table 2 (Continued)

	Placebo (n = 7)	Pemafibrate (n = 11)	P-value (vs placebo)
FGF21 (pg/mL)			
Baseline	382.4 ± 140.6	409.1 ± 179.5	
Week 12	340.7 ± 143.9	866.8 ± 451.0	
Change	−41.7 ± 37.4	457.7 ± 402.1	0.007
Adiponectin (μg/mL)			
Baseline	2.71 ± 1.07	2.71 ± 1.08	
Week 12	3.05 ± 1.15	2.65 ± 0.61	
Change	0.34 ± 0.62	−0.06 ± 0.63	0.131
AST (U/L)			
Baseline	27 ± 10	22 ± 7	
Week 12	29 ± 10	26 ± 10	
Change	1 ± 6	4 ± 7	0.513
ALT (U/L)			
Baseline	41 ± 24	28 ± 11	
Week 12	42 ± 18	25 ± 12	
Change	1 ± 13	−3 ± 11	0.138
GGT (U/L)			
Baseline	83 ± 64	57 ± 55	
Week 12	93 ± 79	27 ± 14	
Change	10 ± 19	−30 ± 46	0.009

Data are presented as mean ± standard deviation. ALT, alanine aminotransferase; Apo, apolipoprotein; AST, aspartate aminotransferase; FFA, free fatty acid; FGF21, fibroblast growth factor 21; FPG, fasting plasma glucose; GGT, gamma-glutamyl transpeptidase; HbA$_{1c}$, glycated hemoglobin; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostatic model assessment for insulin resistance; TG, triglyceride.

DISCUSSION

In patients with hypertriglyceridemia and insulin resistance, pemafibrate treatment (0.2 mg b.i.d.) for 12 weeks resulted in a significant increase in the SGU rate from baseline ($P < 0.01$), although the difference between the pemafibrate and placebo groups did not reach statistical significance ($P = 0.084$). The changes in the PGU rates were not statistically significant, and no clinically meaningful correlation was found between the SGU or PGU rate and laboratory parameters. Given the significant reduction in the plasma glycoalbumin level, we speculate that the study was slightly underpowered to detect the difference in the SGU and PGU rates and the correlations. Taken together, these findings indicate that pemafibrate might improve insulin resistance on SGU regulation.

The methodology used to measure the SGU rate in the present study has been well validated in other studies using similar techniques[6,28–31] with several assumptions that should be recognized: (i) ingested glucose was completely absorbed; (ii) HGP was completely suppressed; (iii) PGU was not affected by OGL; and (iv) endogenous insulin secretion was not affected by OGL.

Conclusive evidence regarding the effects of PPARα agonists on insulin sensitivity was not established. Bezafibrate decreased
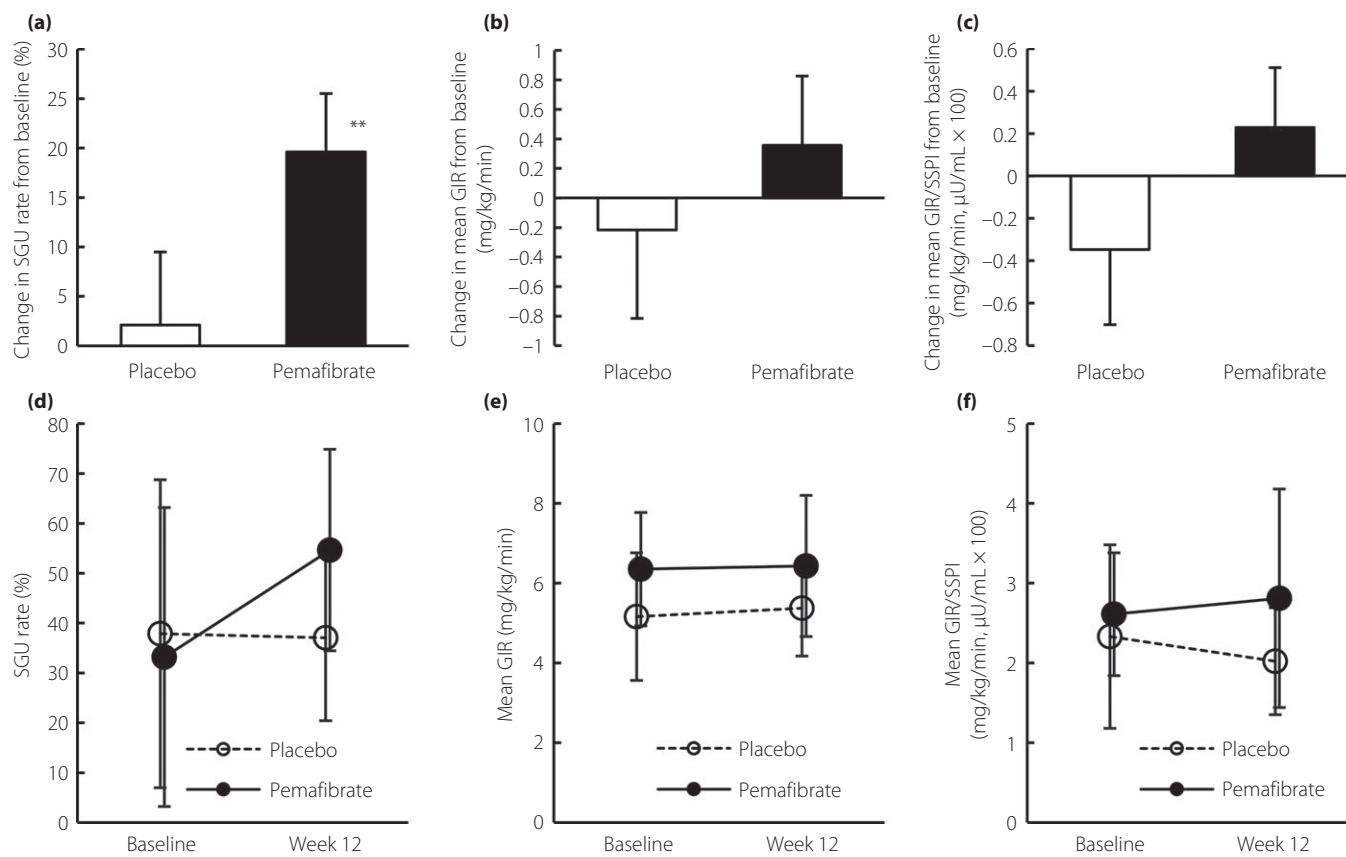
Figure 1 | Changes from (a–c) baseline to week 12 and (d–f) levels at weeks 0 and 12 in the (a, d) splanchnic glucose uptake (SGU) rate, (b, e) mean glucose infusion rate (GIR) and (c, f) mean GIR/steady-state plasma insulin (SSPI). The open and closed symbols indicate the placebo ($n = 7$) and pemafibrate ($n = 11$) groups, respectively. Data are presented as (a–c) the least squares mean ± standard error of the mean estimated using ANCOVA with the baseline value as a covariate or (d–f) the mean ± SD. **$P < 0.01$ versus baseline.

the HOMA-IR and increased the PGU in patients with type 2 diabetes[15,16]. This finding might be attributed to the low PPARα selectivity and partial PPARγ agonistic activity, albeit weak, of bezafibrate[34]. GFT-505, a PPARα/δ dual agonist, improved the insulin-stimulated HGP suppression and PGU in abdominally obese patients[10]. This result could be associated with the PPARδ agonistic activity. In contrast, fenofibrate, which has higher PPARα selectivity than bezafibrate, showed inconsistent effects on insulin sensitivity, and no clinical studies have shown improvement in the insulin-stimulated glucose uptake either in the liver or peripheral tissues[5,14]. Therefore, the suggestion from the present study that pemafibrate, which has even higher PPARα selectivity than fenofibrate, might increase SGU is interesting. A previous phase 2 study[19] and an integrated analysis of phase 2/3 studies[21] showed that the HOMA-IR decreased in patients with elevated HOMA-IR who were treated with pemafibrate in a dose-dependent manner. However, this result was not reproduced in the present study, probably due to the smaller sample size and lower baseline HOMA-IR compared with previous studies.

Next, we investigated the mechanism by which pemafibrate possibly increases SGU and ameliorates insulin resistance.

The improvements in hepatic fat content, liver function tests, and plasma FFA and adiponectin levels have been postulated to mediate the effects of PPARγ agonists on insulin-stimulated glucose disposal and insulin-mediated HGP suppression[5,9].

Although hepatic fat content was not measured in the present study, hepatic TG content decreased in Zucker fatty rats, and the β-oxidation-related gene expression in the liver of Zucker fatty rats and mice, as well as primary human hepatocytes, increased after pemafibrate treatment[18,35]. The gamma-glutamyl transpeptidase level significantly decreased and the ALT level decreased, although this change was not statistically significant, in the present study. Previous studies on the effects of pemafibrate reported significant decreases in both gamma-glutamyl transpeptidase and ALT levels[19,36]. Patients with non-alcoholic fatty liver disease (NAFLD) have higher HOMA-IR and/or lower GIR levels than those without NAFLD[37–39]. Therefore, pemafibrate can possibly ameliorate the potentially impaired liver function, which is a predisposing factor for NAFLD. However, further studies are required, as in the present study the diagnosis of NAFLD was not predetermined and hepatic fat content was not measured.
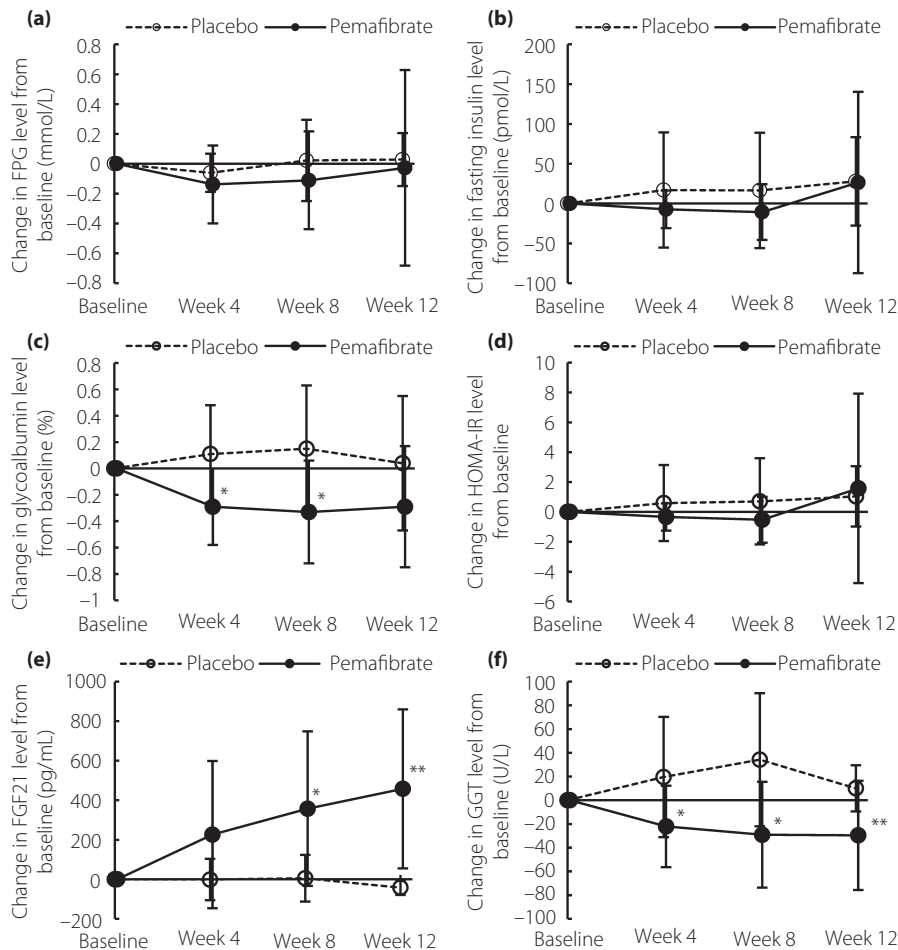
Figure 2 | Changes from baseline over time in the (a) fasting plasma glucose (FPG), (b) fasting insulin, (c) glycoalbumin, (d) homeostatic model assessment for insulin resistance (HOMA-IR), (e) fibroblast growth factor 21 (FGF21) and (f) gamma-glutamyl transpeptidase (GGT) levels. The open and closed circles indicate the placebo ($n = 7$) and pemafibrate ($n = 11$) groups, respectively. Data are presented as mean ± standard deviation. *$P < 0.05$, **$P < 0.01$ versus placebo.

In a previous study, the SGU rate was reduced through exogenous lipid infusion to maintain elevated FFA levels during the hyperinsulinemic-euglycemic clamp test in patients with type 2 diabetes[40]. Therefore, decreased plasma FFA as a result of pemafibrate treatment might contribute, at least in part, to the increase in the SGU rate. However, HGP was stimulated through lipid infusion in the relatively insulinopenic-hyperglycemic clamp method, but not in the hyperinsulinemic-euglycemic clamp method, where HGP was almost completely suppressed. Nevertheless, PGU was suppressed by the lipid infusion in these studies[40,41]. Furthermore, FFA is well documented to compromise the insulin sensitivity in the skeletal muscles rather than that in the liver[24]. Therefore, the decrease in the FFA level is unlikely to make a major contribution on the increase in the SGU on treatment with pemafibrate. In this regard, the effects of pemafibrate on plasma FGF21 are noteworthy.

FGF21 is a novel member of the FGF family with different metabolic effects, including decreased blood glucose and plasma lipid levels. FGF21 expression is induced through starvation and ketogenic diets through the PPARα signaling pathway[25,42]. Exogenous FGF21 administration in *ob/ob* mice restored the insulin-mediated HGP suppression along with the increase in glucokinase activity and hepatic glycogen content, although the glucose 6-phosphatase activity remains unaffected, suggesting that the net flux of glucose in the liver favored glucose uptake[43]. Pemafibrate induced FGF21 expression in Zucker fatty rats and humans more potently than fenofibrate[19,35]. Therefore, a pemafibrate-induced increase in plasma FGF21 might play a role in the mediation of insulin-dependent hepatic glucose disposal. However, further studies are required to clarify whether the effects of FGF21 can still be observed under conditions with almost complete HGP suppression in a hyperinsulinemic state.

The adiponectin level did not increase on treatment with pemafibrate compared with the placebo in the present study. However, fenofibrate increased the adiponectin receptor expression and improved the adiponectin signaling in mice[44]. GFT-505 improved the hepatic insulin sensitivity and liver function test, but reduced the adiponectin levels compared with the placebo. Therefore, the investigators suggested that GFT-505 might improve adiponectin signaling[10].

Plasma FFA and FGF21 also play a role in the mediation of peripheral insulin sensitivity[24,25]. However, it remains to be established whether the significant changes in the FFA and FGF21 levels induced by pemafibrate had an impact on PGU.

The present study had several limitations. First, the number of patients in the per-protocol set was lower than the estimated number to have sufficient statistical power, because a considerable number of patients were excluded from 44 patients who provided informed consent; 17 were not eligible and nine were excluded from the per-protocol set as described above. Second, considering that the study participants were all Japanese and men, the results might not be generalizable to other populations. Third, HGP was not evaluated in the present study. However, according to the mean SSPI, residual HGP was assumed to be negligible in the calculation of the SGU rate. Fourth, even though the methodology used to measure the SGU rate in the present study has been well validated in other studies using similar techniques[6,28–31], as discussed above, there is no guarantee that all of the assumptions were well satisfied in the present study. Specifically, endogenous insulin secretion was not completely suppressed by exogenous insulin infusion and could be stimulated by OGL even under the hyperinsulinemic clamp, which might have confounding effects on insulin-mediated glucose production and uptake in the liver.

In conclusion, pemafibrate increased SGU from baseline in patients with hypertriglyceridemia, possibly through the stimulation of FA β-oxidation and improvement of liver function. FGF21 might also be involved in this mechanism.

ACKNOWLEDGMENTS

DISCLOSURE

REFERENCES

1. Martin BC, Warram JH, Krolewski AS, et al. Role of glucose and insulin resistance in development of type 2 diabetes mellitus: results of a 25-year follow-up study. Lancet 1992; 340: 925–929.

2. DeFronzo RA. Insulin resistance, lipotoxicity, type 2 diabetes and atherosclerosis: the missing links. The Claude Bernard Lecture 2009. Diabetologia 2010; 53: 1270–1287.

3. Robins SJ, Lyass A, Zachariah JP, et al. Insulin resistance and the relationship of a dyslipidemia to coronary heart disease: the Framingham Heart Study. Arterioscler Thromb Vasc Biol 2011; 31: 1208–1214.

4. Desvergne B, Wahli W. Peroxisome proliferator-activated receptors: nuclear control of metabolism. *Endocr Rev* 1999; 20: 649–688.

5. Bajaj M, Suraamornkul S, Hardies LJ, *et al*. Effects of peroxisome proliferator-activated receptor (PPAR)-alpha and PPAR-gamma agonists on glucose and lipid metabolism in patients with type 2 diabetes mellitus. *Diabetologia* 2007; 50: 1723–1731.

6. Kawamori R, Matsuhisa M, Kinoshita J, *et al*. Pioglitazone enhances splanchnic glucose uptake as well as peripheral glucose uptake in non-insulin-dependent diabetes mellitus. AD-4833 Clamp-OGL Study Group. *Diabetes Res Clin Pract* 1998; 41: 35–43.

7. Miyazaki Y, Mahankali A, Matsuda M, *et al*. Improved glycemic control and enhanced insulin sensitivity in type 2 diabetic subjects treated with pioglitazone. *Diabetes Care* 2001; 24: 710–719.

8. Spiegelman BM. PPAR-gamma: adipogenic regulator and thiazolidinedione receptor. *Diabetes* 1998; 47: 507–514.

9. Tiikkainen M, Hakkinen AM, Korsheninnikova E, *et al*. Effects of rosiglitazone and metformin on liver fat content, hepatic insulin resistance, insulin clearance, and gene expression in adipose tissue in patients with type 2 diabetes. *Diabetes* 2004; 53: 2169–2176.

10. Cariou B, Hanf R, Lambert-Porcheron S, *et al*. Dual peroxisome proliferator-activated receptor alpha/delta agonist GFT505 improves hepatic and peripheral insulin sensitivity in abdominally obese subjects. *Diabetes Care* 2013; 36: 2923–2930.

11. Lee CH, Olson P, Hevener A, *et al*. PPARdelta regulates glucose metabolism and insulin sensitivity. *Proc Natl Acad Sci USA* 2006; 103: 3444–3449.

12. Tanaka T, Yamamoto J, Iwasaki S, *et al*. Activation of peroxisome proliferator-activated receptor delta induces fatty acid beta-oxidation in skeletal muscle and attenuates metabolic syndrome. *Proc Natl Acad Sci USA* 2003; 100: 15924–15929.

13. Staels B, Dallongeville J, Auwerx J, *et al*. Mechanism of action of fibrates on lipid and lipoprotein metabolism. *Circulation* 1998; 98: 2088–2093.

14. Black RN, Ennis CN, Young IS, *et al*. The peroxisome proliferator-activated receptor alpha agonist fenofibrate has no effect on insulin sensitivity compared to atorvastatin in type 2 diabetes mellitus; a randomised, double-blind controlled trial. *J Diabetes Complications* 2014; 28: 323–327.

15. Shiochi H, Ohkura T, Fujioka Y, *et al*. Bezafibrate improves insulin resistance evaluated using the glucose clamp technique in patients with type 2 diabetes mellitus: a small-scale clinical study. *Diabetol Metab Syndr* 2014; 6: 113.

16. Taniguchi A, Fukushima M, Sakai M, *et al*. Effects of bezafibrate on insulin sensitivity and insulin secretion in non-obese Japanese type 2 diabetic patients. *Metabolism* 2001; 50: 477–480.

17. Fruchart JC. Selective peroxisome proliferator-activated receptor alpha modulators (SPPARMalpha): the next generation of peroxisome proliferator-activated receptor alpha-agonists. *Cardiovasc Diabetol* 2013; 12: 82.

18. Raza-Iqbal S, Tanaka T, Anai M, *et al*. Transcriptome Analysis of K-877 (a Novel Selective PPARalpha Modulator (SPPARMalpha))-Regulated Genes in Primary Human Hepatocytes and the Mouse Liver. *J Atheroscler Thromb* 2015; 22: 754–772.

19. Ishibashi S, Yamashita S, Arai H, *et al*. Effects of K-877, a novel selective PPARalpha modulator (SPPARMalpha), in dyslipidaemic patients: A randomized, double blind, active- and placebo-controlled, phase 2 trial. *Atherosclerosis* 2016; 249: 36–43.

20. Catapano AL, Graham I, De Backer G, *et al*. 2016 ESC/EAS Guidelines for the Management of Dyslipidaemias: The Task Force for the Management of Dyslipidaemias of the European Society of Cardiology (ESC) and European Atherosclerosis Society (EAS) Developed with the special contribution of the European Assocciation for Cardiovascular Prevention & Rehabilitation (EACPR). *Atherosclerosis* 2016; 253: 281–344.

21. Araki E, Ishibashi S, Yamashita S, *et al*. A highly potent and specific PPAR alpha agonist, K-877, improves lipid profiles and insulin sensitivity in dyslipidaemia subjects; an integrated analysis of 3 Phase 2/3 trials. ePoster #663 presented at EASD 2014 2014 [cited 2016 14 December]. Available from: http://www.easdvirtualmeeting.org/resources/a-highly-potent-and-specific-ppar-alpha-agonist-k-877-improves-lipid-profiles-and-insulin-sensitivity-in-dyslipidaemia-subjects-an-integrated-analysis-of-3-phase-2-3-trials--2.

22. Tripathy D, Almgren P, Tuomi T, *et al*. Contribution of insulin-stimulated glucose uptake and basal hepatic insulin sensitivity to surrogate measures of insulin sensitivity. *Diabetes Care* 2004; 27: 2204–2210.

23. Ruan H, Dong LQ. Adiponectin signaling and function in insulin target tissues. *J Mol Cell Biol* 2016; 8: 101–109.

24. Boden G. Role of fatty acids in the pathogenesis of insulin resistance and NIDDM. *Diabetes* 1997; 46: 3–10.

25. Kliewer SA, Mangelsdorf DJ. Fibroblast growth factor 21: from pharmacology to physiology. *Am J Clin Nutr* 2010; 91: 254s–257s.

26. DeFronzo RA, Tobin JD, Andres R. Glucose clamp technique: a method for quantifying insulin secretion and resistance. *Am J Physiol* 1979; 237: E214–E223.

27. Pilo AFE, Björkman O, Wahren J, *et al*. Analysis of glucose production and disappearance rates following an oral glucose load in normal subjects: a double tracer approach. In: Cobelli CBR (ed). Carbohydrate Metabolism: Quantitative Physiology and Mathematical Modelling. London: John Wiley & Sons, Ltd., 1981; 221–228.

28. Bajaj M, Suraamornkul S, Pratipanawatr T, *et al*. Pioglitazone reduces hepatic fat content and augments splanchnic

glucose uptake in patients with type 2 diabetes. *Diabetes* 2003; 52: 1364–1370.

29. Kawamori R, Morishima T, Ikeda M, *et al.* Effect of strict metabolic control on glucose handling by the liver and peripheral tissues in non-insulin-dependent diabetes mellitus. *Diabetes Res Clin Pract* 1994; 23: 155–161.

30. Ludvik B, Nolan JJ, Roberts A, *et al.* A noninvasive method to measure splanchnic glucose uptake after oral glucose administration. *J Clin Invest* 1995; 95: 2232–2238.

31. Sato F, Tamura Y, Watada H, *et al.* Effects of diet-induced moderate weight reduction on intrahepatic and intramyocellular triglycerides and glucose metabolism in obese subjects. *J Clin Endocrinol Metab* 2007; 92: 3326–3329.

32. Basu R, Basu A, Johnson CM, *et al.* Insulin dose-response curves for stimulation of splanchnic glucose uptake and suppression of endogenous glucose production differ in nondiabetic humans and are abnormal in people with type 2 diabetes. *Diabetes* 2004; 53: 2042–2050.

33. Tamura Y, Tanaka Y, Sato F, *et al.* Effects of diet and exercise on muscle and liver intracellular lipid contents and insulin sensitivity in type 2 diabetic patients. *J Clin Endocrinol Metab* 2005; 90: 3191–3196.

34. Tenenbaum A, Fisman EZ. Balanced pan-PPAR activator bezafibrate in combination with statin: comprehensive lipids control and diabetes prevention? *Cardiovasc Diabetol* 2012; 11: 140.

35. Inokuchi Y, Goto S, Takizawa T, *et al.*, editors. K-877, a highly potent and selective PPARa modulator, increases plasma FGF21 and improves triglyceride metabolisms in Zucker fatty rats. ISA 2015; 2015; Amsterdam.

36. Arai H, Yamashita S, Yokote K, *et al.* Efficacy and safety of K-877, a novel selective peroxisome proliferator-activated receptor alpha modulator (SPPARMalpha), in combination with statin treatment: two randomised, double-blind, placebo-controlled clinical trials in patients with dyslipidaemia. *Atherosclerosis* 2017; 261: 144–152.

37. Marchesini G, Brizi M, Bianchi G, *et al.* Nonalcoholic fatty liver disease: a feature of the metabolic syndrome. *Diabetes* 2001; 50: 1844–1850.

38. Marchesini G, Brizi M, Morselli-Labate AM, *et al.* Association of nonalcoholic fatty liver disease with insulin resistance. *Am J Med* 1999; 107: 450–455.

39. Zelber-Sagi S, Nitzan-Kaluski D, Halpern Z, *et al.* Prevalence of primary non-alcoholic fatty liver disease in a population-based study and its association with biochemical and anthropometric measures. *Liver Int* 2006; 26: 856–863.

40. Tomita T, Yamasaki Y, Kubota M, *et al.* High plasma free fatty acids decrease splanchnic glucose uptake in patients with non-insulin-dependent diabetes mellitus. *Endocr J* 1998; 45: 165–173.

41. Ferrannini E, Barrett EJ, Bevilacqua S, *et al.* Effect of fatty acids on glucose production and utilization in man. *J Clin Invest* 1983; 72: 1737–1747.

42. Galman C, Lundasen T, Kharitonenkov A, *et al.* The circulating metabolic regulator FGF21 is induced by prolonged fasting and PPARalpha activation in man. *Cell Metab* 2008; 8: 169–174.

43. Berglund ED, Li CY, Bina HA, *et al.* Fibroblast growth factor 21 controls glycemia via regulation of hepatic glucose flux and insulin sensitivity. *Endocrinology* 2009; 150: 4084–4093.

44. Haluzik MM, Lacinova Z, Dolinkova M, *et al.* Improvement of insulin sensitivity after peroxisome proliferator-activated receptor-alpha agonist treatment is accompanied by paradoxical increase of circulating resistin levels. *Endocrinology* 2006; 147: 4517–4524.